REMARKS

Amendments to the Claims

The Applicants respectfully ask the Examiner to replace all prior versions and listings of claims in the present application with the listing of claims currently provided. Claims 149-175 are new, Claims 4, 61-63, 102, 103, 119-122, 126 and 142-148 were amended, and Claims 65-67 and 123-125 were canceled. Claims 1-3, 9-44, 54 and 68-95 were canceled previously.

Support for the BoNT/A substrate recited in claim 4 and the claims depending from this independent claim can be found throughout the present specification including, *e.g.*, in Example 1 on pg. 29, para. 191 of US Patent Publication 2003/0143651, which illustrates, in part, a wide range of peptides where the donor fluorophore and acceptor are separated by at least 14 amino acids; on pg. 3, para. 21 and pg. 5, para. 141 of US Patent Publication 2003/0143651, which discloses, in part, that different numbers of residues can separate donor fluorophores from acceptors and provides non-limiting example ranges of 6 to 40 residues; and on pg. 3, para. 21 and pg. 5, para. 141 of US Patent Publication 2003/0143651, which discloses, in part, that a Clostridial toxin substrate can be a variety of lengths.

Support for the BoNT/A substrate recited in claim 102 and the claims depending from this independent claim can be found throughout the present specification including, *e.g.*, on pg. 23, para. 149 of US Patent Publication 2003/0143651, which discloses, in part, embodiments that can exclude fluorophores from the active site cavity of a toxin and non-limiting aspects of this embodiment which teach that donor fluorophores and acceptors can be excluded from residues Arg191 and Met202 of SNAP-25; in Example 1 on pg. 29, para. 191 of US Patent Publication 2003/0143651, which illustrates, in part, a wide range of peptides where the acceptor is excluded from residues Arg191 and Met202 of SNAP-25; on pg. 3, para. 21 and pg. 5, para. 141 of US Patent Publication 2003/0143651, which discloses, in part, that different numbers of residues can separate donor fluorophores from acceptors and provides non-limiting example ranges of 6 to 40 residues; and on pg. 3, para. 21 and pg. 5, para. 141 of US Patent Publication 2003/0143651, which discloses that a Clostridial toxin substrate can be a variety of lengths.

Support for the BoNT/A substrate recited in claim 126 and the claims depending from this independent claim can be found throughout the present specification including, *e.g.*, on pg. 22, para. 139 of US Patent Publication 2003/0143651, which discloses, in part, that donor fluorophores or acceptors can be genetically encoded polypeptides and non-limiting aspects of this embodiment which disclose BFP, CFP, GFP, YFP and RFP; and on pg. 3, para. 21 and pg. 5, para. 141 of US Patent Publication 2003/0143651, which discloses, in part, that a Clostridial toxin substrate can be a variety of lengths.

Rejection Pursuant to 35 U.S.C. §103(a) Obviousness

The Examiner has rejected Claims 4-8, 45-53, 57-67 and 96-148 as allegedly being obvious under 35 U.S.C. §103(a) over three references:

1) James J. Schmidt and Robert G. Stafford, *High Throughput Assays for the Proteolytic Activities of Clostridium Neurotoxins*, U.S. Patent 6,762,280 (Jul. 13, 2004), hereafter the Schmidt patent.

2) B. P. Holskin et al., *A Continuous Fluorescence-based Assay of Human Cytomegalovirus Protease Using a Peptide Substrate*, 226 (1) Anal. Biochem. 148-155 (1995), hereafter the Holskin reference.

3) Nupam P. Mahajan et al., *Novel Mutant Green Fluorescent Protein Protease Substrates Reveal the Activation of Specific Caspases During Apoptosis*, 6(6) Chem. Biol. 401-409 (1999), hereafter the Mahajan reference.

Specifically, the Examiner contends that it would have been obvious to a person of ordinary skill in the art at the time the invention was made to combine the teaching of the Schmidt patent with the Holskin reference to prepare a Clostridial toxin substrate containing donor and acceptor fluorophores as presently claimed in Claim 4 and its dependent claims and Claim 102 and its dependent claims. The Examiner also argues that the combined teaching of the Schmidt patent in view of the Mahajan reference makes obvious a Clostridial toxin substrate containing genetically encoded donor and acceptor fluorophores as presently claimed in Claim 126 and its dependent claims. The Applicants respectfully ask for reconsideration under 37 C.F.R. §1.111.

The prior art references cited teaches away from the claimed invention of Claim 4 and its dependent claims.

With respect to Claims 4-8, 45-53, 57-67 and 96-101, the Applicants respectfully submit that the BoNT/A substrate comprising a donor fluorophore and acceptor of Claim 4 and the claims depending from this independent claim recite, in part, "wherein at least 14 amino acids separate said donor fluorophore from said acceptor."

According to *MPEP* §2143, to render a pending claim obvious, a reference must expressly or impliedly teach or suggest the claimed subject matter. The Applicants respectfully submit that there is a *per se* demonstration of lack of *prima facie* obviousness because the Schmidt patent and the Holskin reference teach away from a BoNT/A substrate having at least 14 amino acids separating the donor fluorophore from the acceptor as presently claimed.

Using a 12 amino acid peptide substrate, the Holskin reference teaches away from using more than 11 amino acids to separate a donor fluorophore from an acceptor fluorophore. Holskin discloses that the "separation distance between the donor/acceptor is essential for efficient resonance energy transfer," see, pg. 154, col. 1, para. 1, lines 1-2. The Holskin reference then proceeds to illustrate this point by teaching that resonance energy transfer is dramatically reduced when the amino acids that separate the donor and acceptor fluorophores are increased from 8 to 11 residues. First this reference indicates that the DABCYL/EDANS fluorophore pair has a calculated Förster distance for 50% energy transfer of about 33 angstroms. The Holskin reference then shows that when 8 amino acids separate the donor fluorophore from the acceptor fluorophore, an estimated distance of about 29 angstroms, a 34-40-fold increase in fluorescence quantum yield is detected, see, *e.g.*, pg. 154, col. 1, para. 1, lines 5-10. However, only a 10-fold increase in fluorescence

quantum yield is detected when 11 amino acids separate the donor and acceptor fluorophores, an estimated distance of about 40 angstroms, see, *e.g.*, pg. 154, col. 1, para. 1, lines 15-21. Taken together, this reference clearly indicates that increasing the distance separating the two fluorophores beyond 11 amino acids would only continue to exponentially decrease the effectiveness of resonance energy transfer. Thus, the Holskin reference expressly teaches that using more than eleven amino acids would be ineffective.

In support of this view, the two 17 amino acid BoNT/A substrates disclosed in the Schmidt patent have only five amino acids (SEQ ID. NO: 1) or only two amino acids (SEQ ID. NO: 2) separating the two fluorophores, see, *e.g.*, col. 5, lines 24-44. In addition, the two BoNT/B substrate and three BoNT/D-F substrates disclosed in this patent also maintain a two to five amino acid separation between donor and acceptor fluorophores, even though these substrates are 35-39 amino acids in length, see, *e.g.*, col. 5, lines 45-67 through col. 6, lines 1-53 and SEQ. ID. NO: 3-7. Thus, in spite of the fact that the donor and acceptor fluorophores could have been theoretically placed further apart from one another in these longer peptide substrates, they were not. Therefore, the Schmidt patent clearly indicates that short separation distances of two to five amino acids between fluorophores were desirable for the disclosed substrates, a teaching consistent with the teaching of the Holskin reference. This teaching has been explicitly confirmed by Schmidt in an article published in 2003, where he writes, "Because the efficiency of energy transfer is inversely proportional to distance between fluorescence donor and acceptor, we wanted to place the two as close as possible in the peptide sequence," see, pg. 298, col. 2, lines 1-4 of James J. Schmidt and Robert G. Stafford, *Fluorigenic Substrates for the Protease Activities of Botulinum Neurotoxins, Serotypes A, B, and F*, 69(1) Appl. Environ. Microbiol. 287-303 (2003).

Similarly, modification of the SEQ ID NO:11 and SEQ ID NO: 12 substrates in the manner suggested by the Examiner, would also not be obvious over the BoNT/A substrates presently claimed. This is because the teaching of the Holskin reference and Schmidt patent would direct one of ordinary skill in the art to maintain short separation distances of only two to five amino acids between the donor and acceptor fluorophore.

This short separation distance is not surprising because it was known by one of ordinary skill at the time that a linear increase in the distance between the donor and acceptor fluorophores results in an exponential decrease in energy transfer between the fluorophores and thus, an exponential decrease in fluorescence quantum yield detected. See also the Mahajan reference which similarly discusses throughout the requirement of short separation distances between donor and acceptor fluorophores of about 10 to 50 angstroms and specifically teaches a 4 amino acid separation between fluorophores, see, *e.g.*, on pg. 402, col. 2, para. 1, lines 2-4; on pg. 403, col. 1, para. 1, lines 1-5, pg. 403, col. 2, para. 2, lines 1-5.

Thus, the Schmidt patent and Holskin reference teach that large separation distances between donor fluorophore and acceptor are not desirable for FRET-based energy transfer and specifically point out that an increase from 8 to 11 amino acids led to undesirable results. Therefore, a *prima facie* obviousness case cannot be made because these references teach away from the claimed BoNT/A substrate of Claim 4 and its dependent claims, which recites that at least 14 amino acids must separate the donor fluorophore and acceptor. The Applicants respectfully submit that the Examiner's rejection is unsupported

by the art and respectfully request withdrawal of the 35 U.S.C. §103(a) obviousness rejection for Claims 4-8, 45-53, 57-67 and 96-101.

The prior art references cited teaches away from the claimed invention of Claim 102 and its dependent claims.

With respect to Claims 102-148, the Applicants respectfully submit that the BoNT/A substrate comprising a donor fluorophore and acceptor of Claim 102 and the claims depending from this independent claim recite, in part, "wherein said acceptor is not positioned within SEQ ID NO: 2." The sequence of SEQ ID NO: 2 is a 12 amino acid sequence comprising Arg191 to Met202 of SNAP-25.

As mentioned above, the *MPEP* §2143 indicates that to render a pending claim obvious, a reference must expressly or impliedly teach or suggest the claimed subject matter. The Applicants respectfully submit that there is a *per se* demonstration of lack of *prima facie* obviousness because the Schmidt patent alone, or in combination with the Holskin reference, teaches away from a BoNT/A substrate that excludes an acceptor fluorophore from Arg191 to Met202 of SNAP-25 as presently claimed.

The Schmidt patent discloses 17 amino acid BoNT/A substrates where an acceptor fluorophore (Z or dcC) is located at amino acid position 200 of SNAP-25, see, *e.g.*, col. 5, lines 24-44 and SEQ. ID. NO: 1-2.

Amino Acid Position																	SEQ ID NO:
187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	
S	N	R	T	R	I	D	**X**	A	N	Q	R	A	**Z**	R	M	L	1
S	N	R	T	R	I	D	E	A	N	**X**	R	A	**dcC**	R	M	L	2

While suggesting that additional signaling acceptor fluorophores can be used, this patent teaches that these fluorophores need to be located within Arg191 to Met202 of SNAP-25, see, *e.g.*, col. 6, lines 54-67. For example, the Schmidt patent teaches that the donor fluorophore must be on one side of the cleavage site and the acceptor fluorophore must be on the other side of this site, see, *e.g.*, col. 6, lines 55-59. This excludes placement of the acceptor fluorophore from Ser187 to Gln197 because the cleavage site is Gln197-Arg198. In addition, the Schmidt patent teaches that Arg198 cannot contain a fluorophore because any alteration in this position eliminates the ability of BoNT/A to cleave the substrate, see, *e.g.*, Table I and col. 16, lines 51-65 of James J. Schmidt and Karen A. Bostian, *Assays for the Proteolytic Activities of Serotype A from Clostridium botulinum*, U.S. Patent 5,965,699 (Oct. 12, 1999), hereafter the '699 patent, which was incorporated by reference by the Schmidt patent. Of the remaining positions, only positions 199 and 200 appear suitable to incorporate an acceptor fluorophore. One reason for this is because the Schmidt patent teaches that positions 194 and 197 appear to be the only two suitable sites for the donor fluorophore, since alterations in positions 193, 195 and 196 eliminate the ability of BoNT/A to cleave the substrate, see Table I and col. 16, lines 51-67 through col 17, lines 1-56 of the '699 patent. In conjunction with this reason, as discussed above, the Schmidt patent teaches the desirability of only having two to five amino acids separating the two fluorophores. This means that the donor fluorophore cannot be placed at positions 191 and

192 because this distance would be over 5 amino acids from the closest possible acceptor fluorophore location at position 199. Finally, the Schmidt patent specifically teaches the placement of the acceptor fluorophore at position 200. Because the Schmidt patent indicates that modifications to the disclosed substrates are not straightforward due to the complex and stringent limitations of their substrates, see, *e.g.*, col. 7, lines 1-12 of the Schmidt patent, the placement of an acceptor fluorophore at position 199 or 202, or anywhere else for that matter, would not be obvious because there is no reasonable expectation of success.

Similarly, modification of the SEQ ID NO:11 and SEQ ID NO: 12 substrates in the manner suggested by the Examiner, would also not be obvious over the BoNT/A substrates presently claimed. This is because the teaching of the Schmidt patent would direct one of ordinary skill in the art to place the acceptor fluorophore at position 200.

The Holskin reference simply does not address this feature, and therefore does not suggest, teach or provide motivation to exclude a donor fluorophore from Arg191 to Met202 of SNAP-25.

Thus, the Schmidt patent and Holskin reference teach that the location of an acceptor fluorophore is within Arg191 to Met202 of SNAP-25. Therefore, a *prima facie* obviousness case cannot be made because these references teach away from the claimed BoNT/A substrate of Claim 102 and its dependent claims, which recite that the position of an acceptor is excluded from Arg191 to Met202 of SNAP-25. The Applicants respectfully submit that the Examiner's rejection is unsupported by the art and respectfully request withdrawal of the 35 U.S.C. §103(a) obviousness rejection for Claims 101-148.

The prior art references provide no motivation to combined references and make the claimed invention of Claim 126 and its dependent claims.

With respect to Claims 126-148, the BoNT/A substrate of Claim 126 and the claims depending from this independent claim recite, in part, "wherein said donor fluorophore or said acceptor is genetically encoded." The Examiner contends that the use of genetically encoded donor and acceptor fluorophores would be a modification within the capability of a person of ordinary skill in the art at the time the invention was made. However, MPEP §2143.01 clearly states that the "fact that the claimed invention is within the capabilities of one of ordinary skill in the art is not sufficient by itself to establish *prima facie* obviousness." The Applicants respectfully submit that a *prima facie* obviousness case fails because the Schmidt patent and the Mahajan reference do not provide motivation, suggestion or teaching that would lead one skilled in the art to specifically make a BoNT/A substrate comprising genetically encoded donor and acceptor fluorophores as presently claimed.

As stated in MPEP §2143.01, "the mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination." The Schmidt patent does not disclose the use of genetically encoded donor and acceptor fluorophores, whereas, the Mahajan reference does not disclose the use of Clostridial toxin substrates. Thus, to establish obviousness based on a combination of the elements disclosed by these references, there must be some motivation, suggestion or teaching of the desirability of making the specific combination that is claimed

by the applicant, namely a Clostridial toxin substrate comprising a genetically encoded donor and acceptor fluorophores. However, no such motivation, suggestion or teaching exists in either reference.

The BoNT/A substrate disclosed in the Schmidt patent not only fails to disclose the use of genetically encoded fluorophores it fails to suggest the desirability of such a modification. In fact, the manner of making the BoNT/A substrates disclosed in this patent specifically precludes the use of genetically encoded fluorophores. In order to incorporate the fluorophores disclosed in the Schmidt patent, the substrates had to rely on chemical synthesis protocols, see, *e.g.*, col. 9, lines 55-64. On the other hand, a BoNT/A toxin substrate containing genetically encoded donor and acceptor fluorescent proteins, as disclosed in the Mahajan reference, would be a peptide of approximately 567 amino acids. The chemical synthesis of such a peptide would not have been possible at the time the present invention was filed. Thus, a person of ordinary skill in the art would find no motivation, suggestion or teaching from the cited references to make a BoNT/A substrate comprising genetically encoded donor and acceptor fluorophores as presently claimed.

The Mahajan reference also provides no motivation or desire to combine the cited references in order to produce the claimed invention. For example, on pg. 401, the Mahajan reference states in the Conclusions section "These substrates allow the spatial activation of specific members of the caspase family to be deciphered ... This technology is also likely to be useful for high-throughput screening of reagents that modulate caspase activity." Likewise this reference states, "The method described here could be adapted to decipher the execution pathway for various other apoptotic stimuli." see, *e.g.*, pg. 407, col. 2, para. 2, lines 5-7. "The assay described here will allow the screening, both at the single cell level and via high-throughput methods, of candidate caspase inhibitors that could be applied clinically to modulate disease pathology." see, *e.g.*, pg. 408, col. 1, para. 1, lines 4-8. At best these passages suggest the assay disclosed in the Mahajan reference can be adapted to other proteases involved in apoptosis. However, the Mahajan reference does not mention or even suggest any other type of protease, let alone specifically Clostridial toxins. It should be noted that Clostridial toxins are non-cytotoxic, *i.e.*, the activity of these toxins does not result in cell death, let alone the programmed cell death initiated by caspase or other apoptotic proteases. Thus, a person of ordinary skill in the art would find no motivation, suggestion or teaching from the cited references to make a BoNT/A substrate comprising genetically encoded donor and acceptor fluorophores as presently claimed.

Identification of each individual part claimed is insufficient to defeat patentability of the whole claimed invention. There must be some motivation, suggestion or teaching of the desirability of making the specific combination of the claimed invention. This is not the case. The Schmidt patent discloses a BoNT/A substrate, but does not provide any motivation, suggestion or teaching to use genetically encoded fluorophores. The Mahajan reference suggests the use of caspase substrates containing genetically encoded fluorophores, but does not provide any motivation, suggestion or teaching to make similar substrates for any other non-caspase protease generally, or Clostridial toxins specifically. Thus, an ordinary person in the art would not have been motivated, suggested or taught to modify or combine the substrate of the Schmidt patent and the substrate of the Mahajan reference and make a Clostridial toxin substrate as presently claimed. Therefore, the Applicants respectfully

submit that the Examiner's rejection is unsupported by the art and respectfully request withdrawal of the 35 U.S.C. §103(a) obviousness rejection for Claims 126-148.

The prior art references demonstrate no reasonable expectation of success to combined references and make the claimed invention of Claim 126 and its dependent claims.

With respect to Claims 126-148, *prima facie* obviousness requires that a reasonable expectation of success be suggested or expressed in the prior art combination. The Applicants respectfully submit that one of ordinary skill in the art would not have had a reasonable expectation of success in combining the Schmidt patent and the Mahajan reference as suggested by the Examiner.

MPEP §2143.02 provides that there can be no *prima facie* obviousness unless there is a reasonable expectation of success, and that "[e]vidence showing there was no reasonable expectation of success may support a conclusion of nonobviousness."

The Schmidt patent discloses that the "introduction of non-natural amino acids and/or bulky aromatic or fluorescent groups [to a Clostridial toxin substrate] would be unlikely to result in a functional substrate," see col 2, lines 26-29 of US 6,762,280. In addition, the Schmidt patent also discloses that the design of FRET substrates for clostridial neurotoxins is difficult and not straightforward and teaches that modifications to his substrates will likely result in a loss of substrate function. For example, the Schmidt patent states on col. 7, para. 1-12:

> The general concept of FRET assays has been known for many proteases. However, knowledge provided by FRET assays for other proteases cannot be applied directly to the development of FRET substrates for clostridial neurotoxin protease activities, due to the extreme substrate specificities, sensitivities to even minor structural changes in substrates, and the very large substrate recognition requirements of the latter enzymes. In view of these complex and stringent limitations, design of FRET substrates for clostridial neurotoxin protease activities, with respect to types of signal and quench moieties and placement within the substrate sequences, is not obvious.

Similar statements of unlikely expectations of success can be found throughout the Schmidt patent, such as, *e.g.*, on col 2, lines 20-23; on col 3, lines 3-12; and on col 3, lines 29-34.

As mentioned above, the Mahajan reference discloses the use of fluorescent protein combinations BFP-GFP and CFP-YFP in a substrate used to measure the activity of caspase-1 and caspase-3. Each of these fluorescent proteins is approximately 275 amino acids in length and approximately 28 kDa. The Examiner's proposed combination is to add a donor fluorescent protein and an acceptor fluorescent protein, as disclosed in the Mahajan reference, to a 17 amino acid substrate, as disclosed in the Schmidt patent. However, one of ordinary skill in the art would consider a fluorescent protein a bulky fluorescent group and such alteration of the Schmidt peptide as a major structural modification.

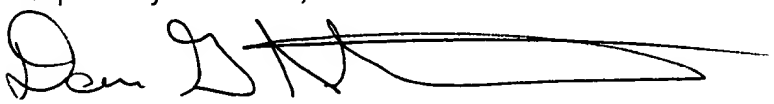
Thus, the Schmidt patent clearly indicates that structural modifications to the substrates disclosed in the '280 patent would render these substrates non-functional and that one of

ordinary skill in the art would consider the addition of fluorescent proteins as donor and acceptor fluorophores as a significant structural modification. Therefore, an ordinary person in the art would not have a reasonable expectation of success in modifying or combining the teachings of the Schmidt patent and the Mahajan reference. The Applicants respectfully submit that this rejection is unsupported by the art and respectfully request withdrawal of the 35 U.S.C. §103(a) obviousness rejection for Claims 126-148.

CONCLUSION

For the above reasons the Applicants respectfully submit that the claims are in condition for allowance, and the Applicants respectfully urge the Examiner to issue a Notice to that effect. Should the Examiner have any questions, he is invited to call the undersigned agent. Please use Deposit Account 01-0885 for the payment of any extension of time fees under 37 CFR §1.136 or any other fees due in connection with the current response.

Respectfully submitted,



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